

University of Groningen

Hospital transfer after a breast cancer diagnosis

Nabon Breast Canc Audit

Published in:
EJSO

DOI:
[10.1016/j.ejso.2018.12.017](https://doi.org/10.1016/j.ejso.2018.12.017)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Final author's version (accepted by publisher, after peer review)

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Nabon Breast Canc Audit (2019). Hospital transfer after a breast cancer diagnosis: A population-based study in the Netherlands of the extent, predictive characteristics and its impact on time to treatment. *EJSO*, 45(4), 560-566. <https://doi.org/10.1016/j.ejso.2018.12.017>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Increasing availability of ω -3 fatty acid in the early-life diet prevents the early-life stress-induced cognitive impairments without affecting metabolic alterations

Kit-Yi Yam,* Lidewij Schipper,[†] Kitty Reemst,* Silvie R. Ruigrok,* Maralinde R. Abbink,* Lianne Hoeijmakers,* Eva F. G. Naninck,* Parand Zarekiani,* Annemarie Oosting,[†] Eline M. Van der Beek,^{†,‡} Paul J. Lucassen,* and Aniko Korosi^{*,1}

*Centre for Neuroscience, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands; [†]Danone Nutricia Research, Utrecht, The Netherlands; and [‡]Department of Pediatrics, University Medical Centre Groningen, University of Groningen, Groningen, The Netherlands

ABSTRACT: Exposure to early-life stress (ES) is associated with cognitive and metabolic deficits in adulthood. The role of early nutrition in programming these long-term effects is largely unknown. We focused on essential ω -3 and ω -6 long-chain polyunsaturated fatty acids (LCPUFA) and investigated whether ES affects central and peripheral FA profiles, as well as if and how an early diet with increased availability of ω -3 LCPUFA (*via* lowering ω -6/ ω -3 ratio) protects against ES-induced impairments. ES exposure [limited nesting and bedding paradigm from postnatal day (P)2 to P9] altered central and peripheral FA profiles in mice. An early diet with low ω -6/ ω -3 ratio from P2 to P42 notably prevented the ES-induced cognitive impairments, and the alterations in hippocampal newborn cell survival and in CD68⁺ microglia, without affecting the ES-induced metabolic alterations. Other markers for hippocampal plasticity, apoptosis, and maternal care were unaffected by ES or diet. Our findings highlight the importance of early dietary lipid quality for later cognition in ES-exposed populations.—Yam, K.-Y., Schipper, L., Reemst, K., Ruigrok, S. R., Abbink, M. R., Hoeijmakers, L., Naninck, E. F. G., Zarekiani, P., Oosting, A., Van der Beek, E. M., Lucassen, P. J., Korosi, A. Increasing availability of ω -3 fatty acid in the early-life diet prevents the early-life stress-induced cognitive impairments without affecting metabolic alterations. *FASEB J.* 33, 5729–5740 (2019). www.fasebj.org

KEY WORDS: neurogenesis • cognition • metabolism • long-chain polyunsaturated fatty acids • microglia

Exposure to early-life stress (ES) increases the vulnerability to develop cognitive impairments and metabolic disorders (1–4). Although there is ample evidence for the involvement of maternal sensory stimuli and stress hormones in such programming (5, 6), the role of nutritional factors (2, 7–11) in this is yet largely unexplored.

Because ω -3 and ω -6 long-chain polyunsaturated fatty acids (LCPUFAs) are critical for cognitive (12–19) and metabolic development (20–27), we focus here on α -linolenic acid (ALA, C18:3 ω -3) and linoleic acid (LA, C18:2

ω -6), which are obtained solely from dietary sources and are mostly converted in the liver to docosahexaenoic acid (DHA, C22:6 ω -3) and arachidonic acid (AA, C20:4 ω -6) (28), respectively. Many studies have focused either on overall restriction (29–31) or supplementation (32, 33) with dietary ω -3 LCPUFA; however, the ratio between dietary LA and ALA is a key determinant of ω -3 LCPUFA status because LA and ALA compete for conversion to their respective LCPUFAs by the same enzymes. Therefore, the shift toward an increased intake of dietary LA in our modern society is considered a serious concern, because an increased ω -6/ ω -3 status (34–36) has been associated with both psychopathologies (14, 36–41) and obesity (42). In contrast, reducing the LA/ALA balance optimized LCPUFA status in the offspring's brain (34) and was beneficial to metabolic health status (43).

Little is known about the short- and long-term effects of ES on LCPUFA status in the offspring and whether LCPUFA can modulate ES-induced cognitive and metabolic changes. Few studies have found an increased ω -6/ ω -3 ratio in plasma or the hippocampus during adulthood as a result of maternal separation in rats (44, 45) (see 46). In addition, perinatal ω -3 PUFA deficiency compromised

ABBREVIATIONS: AA, arachidonic acid; ALA, α -linolenic acid; BrdU, 5-bromo-2'-deoxyuridine; CTL, control; DG, dentate gyrus; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ES, early-life stress; FA, fatty acid; FDR, false discovery rate; LA, linoleic acid; LCPUFA, long-chain polyunsaturated fatty acid; MANOVA, multivariate ANOVA; MUFA, monounsaturated fatty acid; MWM, Morris water maze; OL, object location; OR, object recognition; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TG, triglyceride

¹ Correspondence: Centre for Neuroscience, Swammerdam Institute for Life Sciences, Science Park 904, 1098 XH Amsterdam, The Netherlands. E-mail: a.korosi@uva.nl

doi: 10.1096/fj.201802297R

This article includes supplemental data. Please visit <http://www.fasebj.org> to obtain this information.

metabolic outcomes and induced emotional dysfunction after maternal separation (45, 47, 48). So far, however, it remains unknown if ES exposure in mice alters LCPUFA status or if improving ω -3 PUFA availability by manipulation of the LA/ALA diet ratio during early life can modulate the lasting ES-induced cognitive and metabolic impairments.

We explored here the role of PUFAs in ES-induced programming and assessed if chronic ES affects central and peripheral FA status as well as hepatic lipid metabolism in the short and long term. Next, we subjected ES and control (CTL) mice early in life to either a high or low LA/ALA diet from P2 until P42 to reflect the infancy and prepubertal stage of life in mice (49). We hypothesized that dietary exposure to low LA/ALA would protect against the ES-induced cognitive and metabolic alterations. It is interesting to study both cognitive and metabolic aspects because there is evidence that metabolic factors, fat mass, and the released adipokines (e.g., leptin) are implicated in the modulation of cognitive functions (50–53). Indeed, we previously showed that chronic ES in mice lastingly affects the metabolic phenotype, including persistent reduction in fat mass, which correlated with cognitive dysfunction and reduced circulating leptin (2). We further investigated hippocampal proliferation, cell survival, microglial phagocytosis, neuronal plasticity markers, apoptosis, and maternal care as potential (neuro)biologic substrates for ES-induced cognitive outcomes (1, 54–58).

Our studies show for the first time that 1) ES in mice affects FA status both centrally and peripherally, 2) improving ω -3 PUFA availability through a low LA/ALA diet early in life protects against ES-induced cognitive, but not metabolic, impairments at adult age, and 3) the beneficial effects of the diet involve modulation of hippocampal cell survival and microglia.

MATERIALS AND METHODS

Animals and ES paradigm

C57Bl/6J mice (Harlan Laboratories, Venray, The Netherlands) were bred in-house (2). The day litters detected between 9 and 10 AM were assigned P1. Chronic ES based on limited nesting and bedding material was induced as previously described (1, 2, 59) (Fig. 1A). Briefly, at P2, litters were culled to 6 pups/dam (sex ratio of 3:3 or 4:2), randomly assigned to CTL or ES condition, and left undisturbed until P9. CTL dams received standard nesting and bedding material and stressed dams were placed on a fine-gauge stainless-steel mesh positioned 1 cm above the sawdust-covered cage floor and a reduced amount of nesting material. Mice were weaned at P21, and male littermates were group-housed (2–3 animals/cage). All experimental procedures complied with the principles of laboratory animal care, were carried out in compliance with national legislation following the European Union Directive 2010/63/EU for the protection of animals used for scientific purposes, and were approved by the ethics committee for animal experimentation and Animal Welfare Body of the University of Amsterdam.

Diets

In study 1, dams and male offspring (P9-CTL = 7; ES = 10; P180-CTL = 7; ES = 9) were fed a standard grain-based diet (Teklad Global Rodent Diet 2018; Envigo, Supplemental Table S1)

throughout the study. In study 2, all diets (Sniff-Spezialdiäten, Soest, Germany) were semisynthetic, containing a macro- and micronutrient composition according to the AIN-93G-purified diets for laboratory rodents (60). Dams were fed 2 wk before pregnancy with AIN-93G chow, which contains a higher protein and fat content to support growth and development of juvenile offspring and to optimally support maternal nutritional requirements during pregnancy and lactation. At P2, dams and litters were randomly assigned to isocaloric AIN-93G-based diets containing either a high or low ω -6/ ω -3 diet until P42. The FA composition of these diets was carefully formulated to ensure similar total PUFA between the 2 diets, with differences only in LA and ALA. Hence, the high and low ω -6/ ω -3 diet consists of a LA/ALA ratio of 15:1 and 1:1, respectively (Table 1). After P42, all animals were switched to AIN-93M, which provides all nutrients required for the maintenance of adult animals (Fig. 2A). To study the various parameters at different ages for study 2, the following various groups were generated: P9 [CTL = 8, ES = 9 (high ω -6/ ω -3 diet); CTL = 9, ES = 7 (low ω -6/ ω -3 diet)], P42 [CTL = 8, ES = 10 (high ω -6/ ω -3 diet); CTL = 8, ES = 8 (low ω -6/ ω -3 diet)], P180 [CTL = 11, ES = 11 (high ω -6/ ω -3 diet); CTL = 10, ES = 9 (low ω -6/ ω -3 diet)], and P245 [CTL = 9, ES = 9 (high ω -6/ ω -3 diet); CTL = 10, ES = 11 (low ω -6/ ω -3 diet)].

Behavioral observations and testing

In study 2, maternal care was observed and scored in CTL and ES dams during P3–P7 in the dark phase as previously described (1). Behavioral assessment of offspring was carried out in the active phase of the (reversed) light-dark cycle (lights off at 8 AM), recorded by EthoVision, and scored manually using Observer (Noldus, Wageningen, The Netherlands) by an experimenter blinded to the conditions. Mice were handled for 3 d prior to testing. At P42 and P120, mice were exposed to the T-maze (61) (2 d, 3 trials/day; 2 h intertrial interval; each trial <2 min) (Fig. 4A). Furthermore, we conducted a battery of behavioral tests starting at P120 until P180, which included the object recognition (OR) and object location (OL; 24 h intertrial interval; index of memory: time spent exploring novel or relocated object compared with familiar object) as well as the Morris water maze (MWM; 2 cued trials followed by 6 acquisition d and a single probe trial) as previously described (1).

Tissue collection and dissections

In study 1, P9 and P180 male mice were weighed and decapitated without anesthesia; subsequently, trunk blood, stomach milk, liver, and hippocampus were dissected for FA composition or triglyceride (TG) measurements.

In study 2, dams (P21) were weighed and decapitated after isoflurane anesthesia, followed by trunk blood and liver collection to measure FA composition. At P42, -100, and -180, male offspring underwent the dual-energy X-ray absorptiometry scan (Lunar PIXImus; GE Healthcare, Madison, WI, USA) to determine body composition as described earlier (2). Additionally, body weights of each animal and food intake/cage were monitored weekly during P21–P230. At P42 and P180, mice were unfed for 4 h, weighed, and decapitated after isoflurane anesthesia, and trunk blood was collected. Plasma was used to measure leptin and erythrocytes for FA analyses. Stomach milk, liver, hippocampus, and white adipose tissue depots (gonadal, inguinal, mesenteric, perirenal, and retroperitoneal) were dissected, weighed, and stored at -80°C until further analysis. At P210, 4 wk after the last behavioral task, mice were intraperitoneally injected with 5-bromo-2'-deoxyuridine (BrdU, 100 mg/kg, 2×4 d Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands) (Fig. 5A). Four weeks

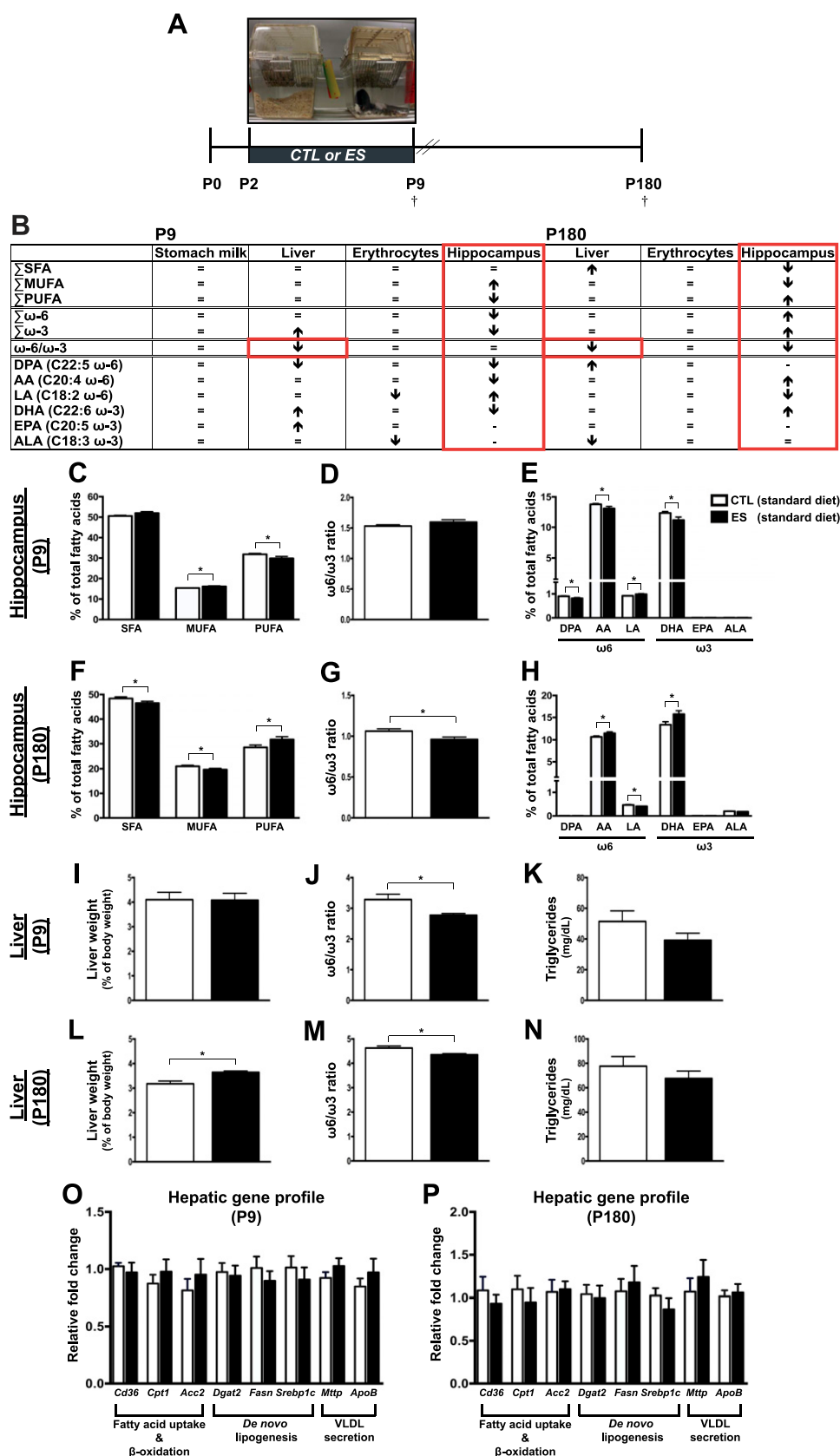


Figure 1. Central and peripheral FA profiles are affected by ES throughout life, whereas hepatic TG and lipid metabolism-related gene expression are unaffected. *A*) Timeline illustrating the ES period from P2 to P9 in CTL and ES mice fed with standard grain-based chow (study 1). † represents the endpoint of mice. *B*) Overview demonstrating the direction of ES effects on the FA composition in central and peripheral tissues at P9 and P180. An overview of the FA values in the stomach milk, liver, erythrocytes, and hippocampus are presented in Supplemental Table S3. *C–E*) Prominent ES effects were found in the hippocampus, showing no differences in the SFA, increased MUFA, and decreased PUFA (*C*); unaltered ω-6/ω-3 ratio (*D*); and decreased DPA and AA, increased LA, and decreased DHA, whereas EPA and ALA were not detectable after ES exposure at P9 (*E*). *F*) At P180, ES decreased SFA and MUFA, whereas PUFA was increased. *G*) Moreover, ES decreased the ω-6/ω-3 ratio. *H*) ES also increased AA, decreased LA, increased DHA, and did not affect ALA; DPA and EPA levels were not detectable. *I–K*) At P9, liver weight was unaltered (*I*), ω-6/ω-3 reduced (*J*), and TG content unaltered (*K*) after ES. *L–N*) At P180, liver weight was increased (*L*), whereas ω-6/ω-3 was decreased (*M*) and TG unaffected (*N*) by ES. *O, P*) ES further did not alter the hepatic lipid metabolism, including processes related to FA uptake and β-oxidation, *de novo* lipogenesis, very low-density lipoprotein secretion at P9 (*O*) and P180 (*P*). Data are presented as means ± SEM; independent 1-sample *t* test with Benjamini Hochberg multiple testing correction for SFA, MUFA, PUFA, DPA, AA, LA, DHA, EPA, and ALA. *FDR < 0.1.

after BrdU injection, at P245, mice were anesthetized (pentobarbital, 120 mg/kg, i.p., Euthasol; ASTfarma, Oudewater, The Netherlands) and perfused transcardially with 4% paraformaldehyde (Sigma-Aldrich Chemie). Perfused brains were

carefully removed and postfixed (4% paraformaldehyde; 24 h). Cryoprotected frozen brains were sliced into 40-μm-thick coronal sections using a microtome, divided over 6 parallel series, and stored at -20°C until use.

TABLE 1. Diet composition of the experimental high and low ω -6/ ω -3 diets (grams/kilogram diet)

Ingredient	High ω -6/ ω -3	Low ω -6/ ω -3
Cornstarch, pregelatinized	397.5	397.5
Casein	200.0	200.0
Maltodextrin 10 DE	132.0	132.0
Sucrose	100.0	100.0
Cellulose	50.0	50.0
Mineral premix	35.0	35.0
Vitamin premix	10.0	10.0
L-cysteine	3.0	3.0
Choline CL (50%)	2.5	2.5
Oil blend	70.0	70.0
Coconut oil, hydrogenated	23.8	23.6
Peanut oil	21.3	20.0
Safflower oil	20.2	6.7
Linseed oil	2.2	19.7
Soybean oil	2.5	—
Fatty acids (% total fatty acids)		
C6:0	0.1	0.1
C8:0	1.9	1.9
C10:0	1.9	1.9
C12:0	15.3	15.4
C14:0	6.9	6.9
C16:0	9.6	9.1
C18:0	6.0	6.3
C20:0	1.0	0.9
Σ SFA	42.6	42.4
C18:1	21.6	22.1
C20:1	0.6	0.4
Σ MUFA	22.1	22.6
C18:2n-6 (LA)	30.6	17.3
C18:3n-3 (ALA)	2.0	15.3
Σ PUFA	32.6	32.6
LA/ALA	15.3	1.1

Leptin, FA composition, and TG analyses

Plasma leptin was assessed at P9, -42, and -180 in study 2 (Milliplex Mouse Adipokine; MilliporeSigma). In both studies 1 and 2, stomach milk, hippocampus, and liver tissues were homogenized and diluted 50 \times in Milli-Q. Lipids from these tissues and erythrocytes were extracted (62), and membrane FA composition was assessed by gas chromatography (percent of total FA). Furthermore, in study 1, TG content was determined by enzymatic colorimetric assays (TG liquicolor; Human Diagnostics, Wiesbaden, Germany) in liver samples at P9 and P180.

Immunohistochemistry and quantification

At P245 in study 2, 3 of the 6 parallel brain series were stained for BrdU (rat- α -BrdU, 1:200/24 h; Accurate Chemical Scientific Corporation, Westbury, NY, USA), Ki67 (rabbit- α -Ki67, 1:20,000/24 h; Leica Microsystems, Buffalo Grove, IL, USA), and CD68 (rat- α -CD68, 1:400/24 h; AbD Serotec, Raleigh, NC, USA) (54) (Fig. 5A). Volume of the granular zone of the dentate gyrus (DG) was determined based on 8 bregma levels (−1.36 to −3.28) as previously described (1). Cells were manually counted in the subgranular and granular zone of the DG for BrdU and Ki67 and expressed per DG and in the whole hippocampus for CD68 (8 bregma levels multiplied by 6; \times 20/40 magnification).

Gene expression analyses

Frozen hippocampal tissues (from study 2) and liver tissues (from study 1) were treated with Trizol (Thermo Fisher Scientific,

Breda, The Netherlands) and cDNA synthesis performed using 250 and 1000 ng RNA, respectively (iScript; Thermo Fisher Scientific). Gene expression of hepatic lipid metabolism, neuronal plasticity, and apoptotic markers were quantified using highly specific primers (Thermo Fisher Scientific; Supplemental Table S2). Stability of 2–3 housekeeping genes was verified using GeNorm (gene stability value <0.5; coefficients variation <0.2) and used for normalization (qBase+3.0; Biogazelle, Ghent, Belgium).

Statistical analyses

Data were analyzed using SPSS 22.2 (IBM Software, Armonk, NY, USA) and graphical design using GraphPad Prism (GraphPad Software, La Jolla, CA, USA). We used the independent and 1-sample *t* test, mixed model 2-way, multivariate or repeated measures ANOVA with Tukey HSD test (when appropriate), and Pearson's correlations (details given in results section). Furthermore, the Benjamini Hochberg was used to correct for multiple testing. The *q* value, also known as the false discovery rate (FDR), was set at a critical value of 0.1 to minimize the chance on false positives. The *z* score for each behavioral task was calculated as $z = (x - \mu) / \sigma$, where *x* is the individual data of the observed parameter, μ is mean, and σ is the SD of the CTL group (CTL+high ω -6/ ω -3) (63). "Hippocampal learning score" is the sum of *z* scores per behavioral test divided by the number of tests. Mice from at least 2 different litters were included in each experimental group, and litter was included as random factor. All data are presented as means \pm SEM and considered statistically significant when *P* < 0.05 (2-tailed) or when below the FDR of 0.1.

RESULTS

ES affects central and peripheral FA profiles throughout life without altering hepatic TG content and lipid metabolism-related gene expression

In study 1, chronic ES (Fig. 1A) altered FA profiles at P9 and P180 in brain and liver (Fig. 1B–N). ES-exposed P9 hippocampus contained higher monounsaturated FA (MUFA; FDR = 0.10) and lower PUFA (FDR trend = 0.11; Fig. 1C), reflected by lower DHA, AA, and DPA (ω -6; all FDR = 0.08), whereas LA was increased (FDR = 0.08; Fig. 1E), and total ω -6/ ω -3 PUFA was unaltered (Fig. 1D). ES led to opposite effects on the FA profile in P180 hippocampus when compared with P9, demonstrating decreased saturated FA (SFA) and MUFA and increased PUFA (all FDR = 0.08; Fig. 1F), accompanied by decreased ω -6/ ω -3 PUFA ($t_{12} = 2.44$, *P* = 0.03; Fig. 1G). Furthermore, ES increased AA (FDR trend = 0.11) and DHA (FDR = 0.09), and LA was decreased (FDR = 0.09; Fig. 1H). Neither total PUFA ($R^2 = 0.04$, *P* = 0.58) nor DHA ($R^2 = 0.00$, *P* = 0.98) showed correlation with OR performances in this P180 cohort (2).

In the liver, ES decreased ω -6/ ω -3 at P9 ($t_{28} = 2.82$, *P* = 0.01; Fig. 1J), including decreased DPA (FDR = 0.00) as well as higher DHA (FDR trend = 0.12) and eicosapentaenoic acid (EPA; FDR = 0.00, Supplemental Table S3), without affecting liver weight (Fig. 1I), hepatic TG content (Fig. 1K), and gene expression related to hepatic lipid metabolism (FA uptake and oxidation, *de novo* lipogenesis, and very low-density lipoprotein secretion) (Fig. 1O). Alterations in

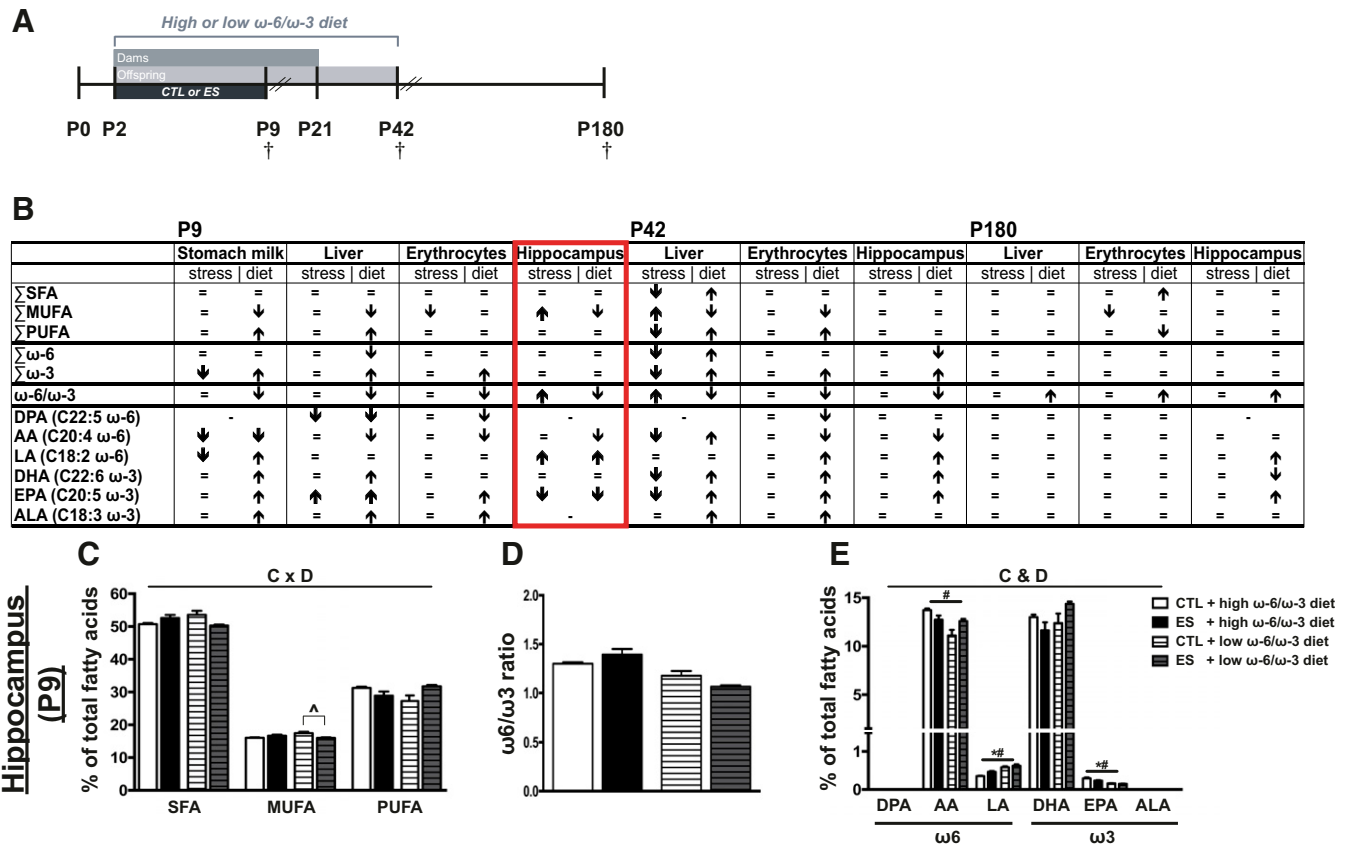


Figure 2. Effects of ES and a high or low ω -6/ ω -3 diet on FA profiles in central and peripheral tissues of P9, -42, and -180 mice. **A)** Timeline illustrating the period of ES from P2 to P9 and dietary intervention with a high or low ω -6/ ω -3 diet from P2 to -42 (study 2). \dagger represents the end-point of mice. **B)** Table overview demonstrating the direction of ES and diet effects on the FA composition in central and peripheral tissues at P9, -42, and -180. An overview of FA values in stomach milk, liver, erythrocytes, and hippocampus are presented in Supplemental Table S4. **C)** In the hippocampus at P9, the SFA and PUFA were unaffected by ES and diet, whereas ES decreased MUFA levels in mice on low ω -6/ ω -3 diet. **D)** Furthermore, ω -6/ ω -3 ratio was unaltered after ES in both diet groups. **E)** Also, AA levels were decreased in the low ω -6/ ω -3 diet group, whereas LA was increased by ES and low ω -6/ ω -3 diet, DHA was unaltered, EPA decreased by ES and low ω -6/ ω -3 diet, and DPA and ALA levels were not detectable. Data are presented as means \pm SEM; MANOVA with SFA, MUFA, PUFA, DPA, AA, LA, DHA, EPA, and ALA; interaction between condition (C) and diet (D) followed by 2-way ANOVA and Tukey *post hoc* test when appropriate; *main effect of condition, #main effect of diet, ^interaction condition*diet. $P < 0.05$.

liver FA profile persisted until P180, as demonstrated by reduced ω -6/ ω -3 ratio in ES ($t_{12} = 2.95$, $P = 0.01$; Fig. 1M), including increased DPA and decreased ALA (both FDR trend = 0.11, Supplemental Table S3). This was accompanied by increased liver weight ($t_{13} = -4.05$, $P < 0.01$; Fig. 1L), with no alterations in hepatic TG content (Fig. 1O) and gene expression related to lipid metabolism (Fig. 1P).

Furthermore, there were no major effects of ES on the FA composition in stomach milk and erythrocytes at P9 and P180 (detailed overview in Supplemental Table S3).

Effects of high and low ω -6/ ω -3 diets on central and peripheral FA profiles after ES

Next, in study 2, we modulated central and peripheral FA profiles after exposure to high or low ω -6/ ω -3 diet (Fig. 2A) and confirmed that maternal low ω -6/ ω -3 diet reduced ω -6/ ω -3 ratio in erythrocytes ($F_{1,15} = 146.06$, $P < 0.01$) and liver ($F_{1,14} = 1166.78$, $P < 0.01$; Supplemental Table S4). The maternal low ω -6/ ω -3 diet also affected P9 offspring FA

status, as shown by lower ω -6/ ω -3 and altered LCPUFA composition in stomach milk, liver, and erythrocytes (Supplemental Table S4).

In the hippocampus, ES-exposed P9 offspring on a high ω -6/ ω -3 diet showed comparable FA composition changes to standard grain-based P9 mice, as described above in study 1 (Fig. 2B–E). ES P9 on low ω -6/ ω -3 diet showed decreased hippocampal MUFA compared with CTL [multivariate ANOVA (MANOVA), condition*diet: $F_{3,20} = 3.40$, $P = 0.04$, HT = 0.51; 2-way ANOVA-MUFA, condition*diet: $F_{1,22} = 8.05$, $P = 0.01$; Fig. 2C]. ES did not affect hippocampal ω -6/ ω -3 (Fig. 2D) but increased LA and decreased EPA in both diet groups (MANOVA, condition: $F_{4,19} = 3.90$, $P = 0.02$, HT = 0.82; diet: $F_{4,19} = 103.94$, $P < 0.01$, HT = 21.88; 2-way ANOVA-LA, condition: $F_{1,22} = 7.03$, $P = 0.02$; diet: $F_{1,22} = 50.30$, $P < 0.01$; EPA, condition: $F_{1,22} = 7.81$, $P = 0.01$; diet: $F_{1,22} = 77.82$, $P < 0.01$; Fig. 2E). Lastly, low ω -6/ ω -3 diet reduced AA regardless of ES ($F_{1,22} = 8.18$, $P = 0.01$; Fig. 2E).

At P42, low ω -6/ ω -3 diet increased PUFA ($F_{1,30} = 5.07$, $P = 0.03$; Supplemental Table S4), LA ($F_{1,30} = 16.76$, $P < 0.01$), DHA ($F_{1,30} = 32.32$, $P < 0.01$), EPA ($F_{1,30} = 265.93$, $P < 0.01$),

and ALA ($F_{1,30} = 157.61$, $P < 0.01$), whereas it decreased ω -6/ ω -3 ratio ($F_{1,30} = 94.13$, $P < 0.01$), MUFA ($F_{1,30} = 26.45$, $P < 0.01$), DPA ($F_{1,30} = 38.36$, $P < 0.01$), and AA ($F_{1,30} = 25.28$, $P < 0.01$) without ES effects in erythrocytes.

We further found interactions between condition and diet on FA compositions in the liver on SFA (MANOVA condition*diet: $F_{3,25} = 4.75$, $P = 0.01$, HT = 0.57; 2-way ANOVA condition*diet: $F_{1,27} = 11.19$, $P < 0.01$; Supplemental Table S4), MUFA ($F_{1,27} = 14.89$, $P < 0.01$), PUFA ($F_{1,27} = 13.58$, $P < 0.01$), AA (MANOVA condition*diet: $F_{6,22} = 4.40$, $P = 0.01$, HT = 1.20; 2-way ANOVA condition*diet: $F_{1,27} = 7.71$, $P = 0.01$), DHA ($F_{1,27} = 15.56$, $P < 0.01$), EPA ($F_{1,27} = 24.98$, $P < 0.01$), and ω -6/ ω -3 ratio ($F_{1,27} = 6.12$, $P = 0.02$).

Furthermore, hippocampal FA composition was only affected by the low ω -6/ ω -3 diet, demonstrating reduced ω -6/ ω -3 ratio ($F_{1,28} = 318.28$, $P < 0.01$) and AA ($F_{1,28} = 45.26$, $P < 0.01$) and increased LA ($F_{1,28} = 87.08$, $P < 0.01$), DHA ($F_{1,28} = 34.23$, $P < 0.01$), and EPA ($F_{1,28} = 16.92$, $P < 0.01$; Supplemental Table S4).

At P180, 20 wk after being switched to AIN-93M chow, there were no effects of ES on FA composition in liver, erythrocytes, and hippocampus and only marginal effects by the early diet (Supplemental Table S4).

ES-induced reductions in adipose tissue deposition and leptin levels are not altered by the diet

In study 2, ES, regardless of dietary exposure, reduced body weight throughout life until P180 (P9: $F_{1,61} = 22.26$, $P < 0.01$; P100: $F_{1,28} = 8.31$, $P < 0.01$; P180: $F_{1,36} = 10.24$, $P < 0.01$; Fig. 3A). This was accompanied by reductions in lean mass (P100: $F_{1,28} = 4.77$, $P = 0.04$; P180: $F_{1,28} = 6.58$, $P = 0.02$; Fig. 3B) and total body fat mass (P180: $F_{1,28} = 13.89$, $P < 0.01$; Fig. 3C).

In line with the dual-energy X-ray absorptiometry measurements in these mice, ES decreased white adipose tissue depot weights at P9 ($F_{1,12} = 12.20$, $P = 0.02$), P42 ($F_{1,29} = 6.94$, $P = 0.01$), and P180 ($F_{1,35} = 6.10$, $P = 0.02$), but not at P245 (Fig. 3D), regardless of diet. The reduction in adiposity was accompanied by reduced circulating leptin at P9 ($F_{1,24} = 8.75$, $P < 0.01$), P42 ($F_{1,21} = 6.68$, $P = 0.02$), and P180 ($F_{1,30} = 4.85$, $P = 0.04$; Fig. 3E) without any diet effects. Furthermore, food intake between P42 and P230 remained unaffected by either ES or diet condition (Fig. 3F).

Low ω -6/ ω -3 diet prevents ES-induced cognitive impairments

In study 2, we found ES-induced impairments in 4-mo-old mice exposed to high ω -6/ ω -3 diet (Fig. 4A–G), confirming previous reported findings under standard grain-based conditions (1, 2, 59). Importantly, low ω -6/ ω -3 diet prevented these ES-induced cognitive impairments in 3 hippocampal-dependent tasks (condition*diet OR: $F_{1,56} = 7.24$, $P = 0.01$; OL: $F_{1,22} = 5.38$, $P = 0.03$; MWM probe, condition: $F_{1,64} = 4.08$, $P = 0.048$; diet: $F_{1,64} = 4.81$, $P = 0.03$; Fig. 4C, E, G), which was further highlighted by the “hippocampal learning score” calculated by combining the z scores of these tasks (condition*diet: $F_{1,45} = 15.13$, $P < 0.01$; Fig. 4J–M). Working memory, as measured by the T-maze at P42 (Fig. 4H) and P120 (Fig. 4I), however, remained unaffected by ES and diet.

Low ω -6/ ω -3 diet prevents the ES-induced reduction in hippocampal cell survival and the increase in the phagocytic marker CD68

In study 2, we showed that chronic ES reduced hippocampal newborn cell survival in adult (P245) mice on high

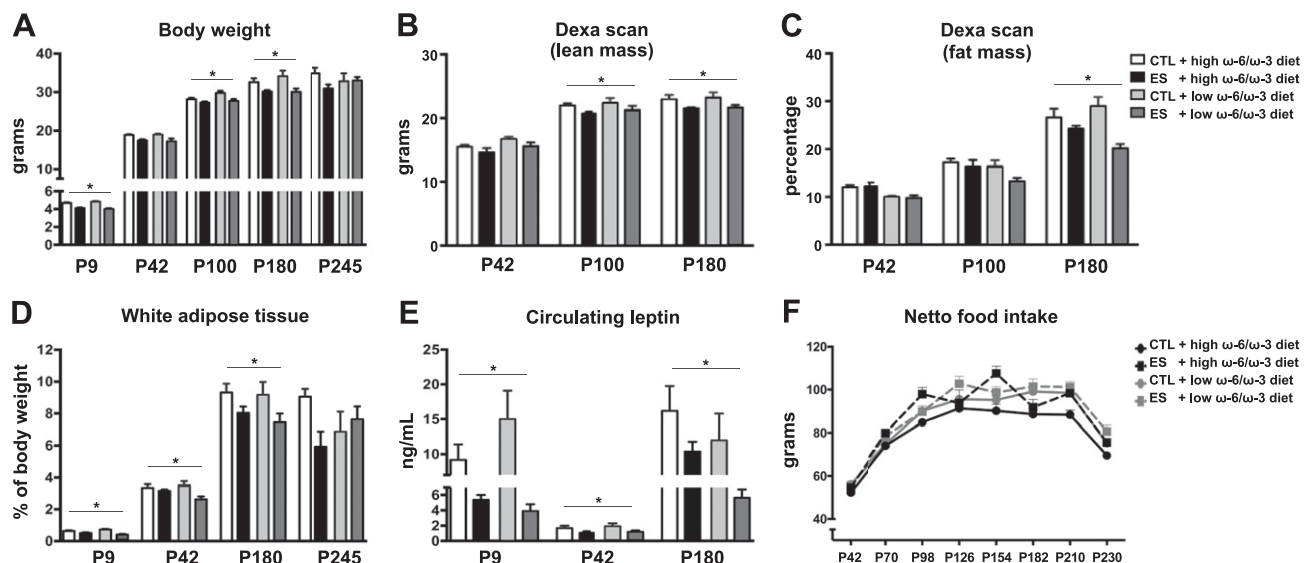


Figure 3. Adiposity and leptin levels in ES-exposed mice are unaffected by the diets throughout life. A–E) ES decreased body weight (A) from P9 until P180, accompanied by decreased lean mass at P100 and P180 (B), and the total fat mass at P180 (C) without diet effects. In addition, ES decreased the amount of white adipose tissues at P9 (inguinal white adipose tissues), P42 (gonadal, inguinal, mesenteric, and retroperitoneal), and P180 (gonadal, inguinal, mesenteric, perirenal, and retroperitoneal) in both diet groups (D) as well as the circulating leptin levels at P9, P42, and P180 (E). F) Moreover, food intake over time from P42 until P230 was unaffected between the experimental groups. DEXA, dual-energy X-ray absorptiometry. Data are presented as means \pm SEM; 2-way ANOVA and Tukey *post hoc* test when appropriate; *main effect of condition. $P < 0.05$.

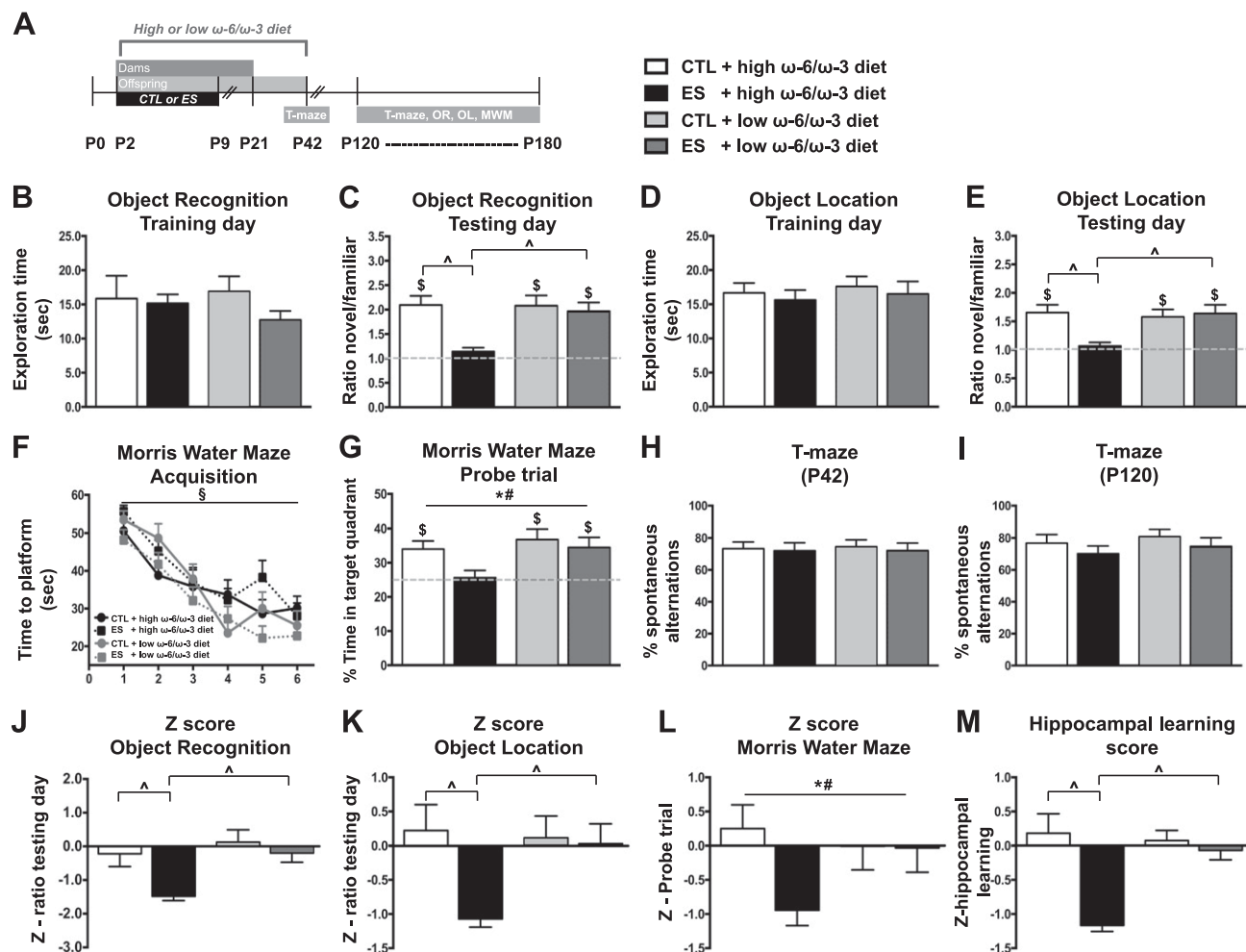


Figure 4. Lasting ES-induced hippocampus-dependent cognitive impairments are prevented by the low ω -6/ ω -3 diet. **A)** Timeline illustrating the time period in which mice were exposed to ES from P2 to P9 and the high or low ω -6/ ω -3 diet from P2 to P42. **B–E)** A battery of behavioral tasks was carried out. On training day, there were no differences in exploration time between the groups in the OR task (**B**). On testing day, ES mice on high ω -6/ ω -3 diet showed impaired learning, whereas ES mice on low ω -6/ ω -3 diet demonstrated improved learning in the OR (**C**). Similarly, there were no differences in exploration time between the groups in the OL task on training day (**D**). And on testing day, ES mice on high ω -6/ ω -3 diet had impaired learning, whereas learning was improved in ES mice on low ω -6/ ω -3 diet in the OL (**E**). **F, G)** Over a time period of 6 d, all mice learned to find the hidden platform in the MWM task (**F**), and ES mice on low ω -6/ ω -3 diet showed improved learning in the probe trial as compared to ES mice on high ω -6/ ω -3 diet (**G**). **H, I)** Spontaneous alternations for 2 consecutive days with 3 trials a day in P42 (**H**) and P120 (**I**) mice were not affected by ES and diet as assessed by the T-maze. **J–M)** Z normalization was calculated for the OR (**J**), OL (**K**), and MWM (**L**) probe trial, resulting in an overall “hippocampal learning score” (**M**). Data are presented as means \pm SEM; 2-way ANOVA and Tukey *post hoc* test when appropriate; *main effect of condition, #main effect of diet, ^interaction condition*diet. One-sample *t* test to compare the performances above chance level; §Repeated measures ANOVA for the acquisition days; §main effect of time. $P < 0.05$.

ω -6/ ω -3 diet (Fig. 5D–F), confirming our earlier findings (1). The low ω -6/ ω -3 diet prevented this reduced cell survival in ES mice ($F_{1,26} = 4.12$, $P = 0.05$; Fig. 5D–F), whereas hippocampal proliferation (Fig. 5B, C) and the DG volume (granular zone; data not shown) were unaffected by ES and the diets. Moreover, ES increased the number of hippocampal microglial CD68⁺ cells in the high ω -6/ ω -3 group as previously shown (54), which was prevented by the low ω -6/ ω -3 diet ($F_{1,16} = 4.94$, $P = 0.04$; Fig. 5G, H). In addition, hippocampal learning score (Fig. 4M) was negatively correlated with microglial CD68 cells ($R^2 = -0.41$, $P < 0.01$; Fig. 5J) but not with newborn cell survival (Fig. 5I).

Expression of hippocampal plasticity markers *Bdnf*, *Synapsin*, and *Psd95* at P9, -42, and -180 in study 2 was mostly unaffected by ES or diet with a few exceptions. *Bdnf* mRNA

was decreased in P9 on low ω -6/ ω -3 diet ($F_{1,16} = 6.09$, $P = 0.03$; Fig. 5K) and was transiently increased in P42 ES mice in the high ω -6/ ω -3 diet group only ($F_{1,13} = 6.73$, $P = 0.02$). No ES or diet effects were found on *Bdnf* expression at P180.

Moreover, hippocampal ratio of *Bax* (proapoptotic) and *Bcl2* (antiapoptotic) mRNA was reduced at P180 by ES without diet effects ($F_{1,35} = 10.72$, $P < 0.01$; Fig. 5L), whereas at P9 and P42, no effects of ES or diet were detected.

Diet does not affect the ES-induced effects on maternal care, body weight, and food intake

In study 2, ES-induced fragmented maternal care (e.g., increased time of pups out of nest: high ω -6/ ω -3 group,

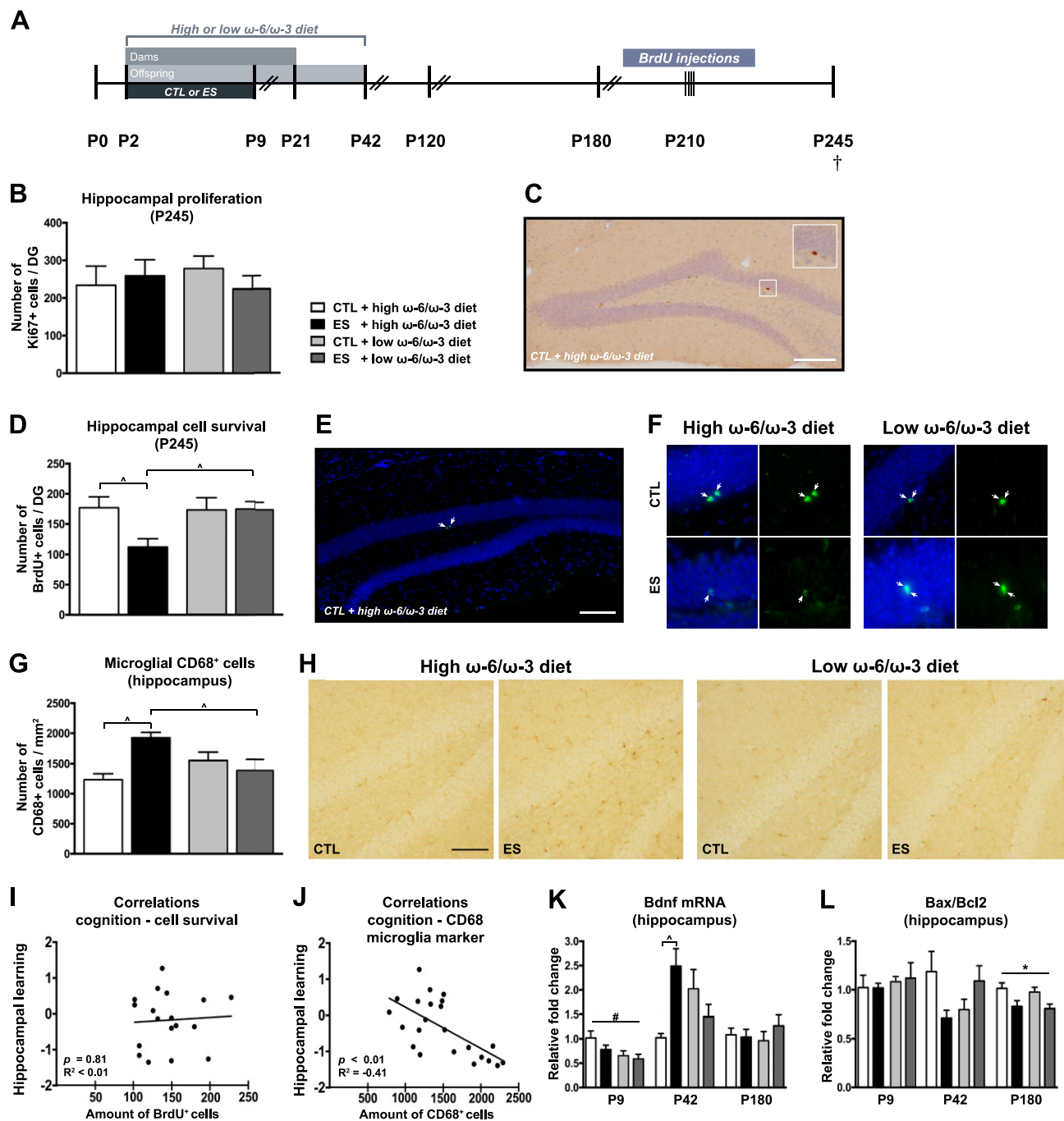


Figure 5. ES-induced alterations on hippocampal cell survival and the phagocytic CD68 marker are prevented by the low ω -6/ ω -3 diet. *A,B*) Timeline (*A*) showing the ES from P2 to P9 and the high or low ω -6/ ω -3 diet from P2 to P42. Mice were injected with BrdU 1 mo after behavior at P210 to assess hippocampal cell survival in P245 mice, in which hippocampal proliferation (*B*) was unaltered by ES and diet. † represents the end-point of mice. *C*) Representative picture of the DG containing Ki67⁺ cells. *D*) ES-induced reduction in hippocampal cell survival was prevented by the low ω -6/ ω -3 diet. *E, F*) Representative picture of the DG containing BrdU⁺ cells. *G*) Moreover, ES-induced increases in CD68 were prevented by the low ω -6/ ω -3 diet. *H*) Representative picture of CD68⁺ cells of CTL and ES mice on high and low ω -6/ ω -3 diet. *I, J*) No correlation was found between hippocampal learning z score and cell survival (*I*), whereas it was negatively correlated with the amount of CD68⁺ cells (*J*). *K*) Hippocampal *Bdnf* expression was decreased by the low ω -6/ ω -3 diet at P9, and ES increased *Bdnf* only in P42 ES-exposed mice in the high ω -6/ ω -3 diet group. *L*) ES reduced the Bax/Bcl2 ratio in P180 mice in both diet groups. Scale bars represent 100 μ m. Data are presented as means \pm SEM; 2-way ANOVA and Tukey *post hoc* test when appropriate; *main effect of condition, #main effect of diet, ^interaction condition*diet. $P < 0.05$.

$F_{1,9} = 28.61$, $P < 0.01$; low ω -6/ ω -3 group, $F_{1,10} = 34.75$, $P < 0.01$; **Fig. 6A**; number of dam nest exits: high ω -6/ ω -3 group, $F_{1,6} = 45.00$, $P < 0.01$; low ω -6/ ω -3 group, $F_{1,9} = 45.77$, $P < 0.01$; **Fig. 6B**; no effect on time spent nursing; **Fig. 6C**). In addition, ES decreased

body weight gain in dams between P2 to P9 ($F_{1,39} = 4.27$, $P = 0.46$; **Fig. 6D**) and increased food intake ($F_{1,39} = 21.42$, $P < 0.01$; **Fig. 6E**) without affecting water intake (**Fig. 6F**). There were no effects of diet on these parameters.

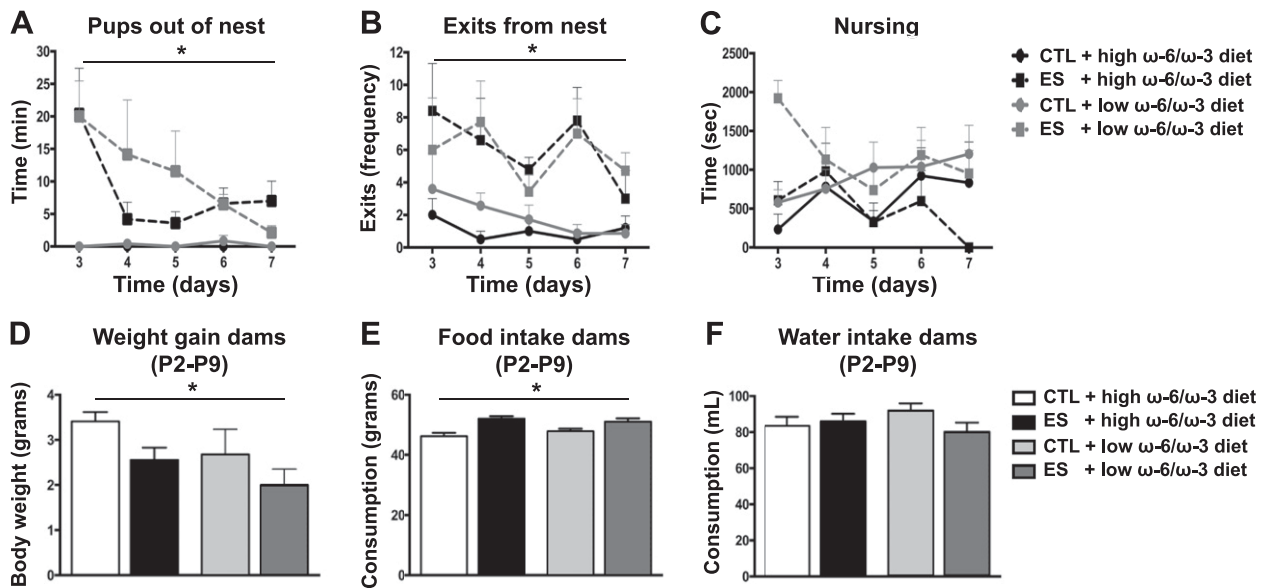


Figure 6. A, B) ES-induced fragmented maternal care, altered body weight, and food intake are not affected by the diet. ES-induced fragmented maternal care as assessed by the increased time of pups out of nest (A) and increased frequency of exits from the nest by dams (B) in both diet groups throughout P3 until P7. C–F) Nursing time (C) was unaffected by both ES and diet. From P2 to P9, ES dams showed reduced body weight gain (D), increased food intake (E), and unaffected water intake (F). Data are presented as means \pm SEM; repeated measures ANOVA; *main effect of time. $P < 0.05$.

DISCUSSION

ES exposure has a lasting impact on cognition and metabolism. Our study showed that ES alters the central FA profile of the offspring and that restoring this profile *via* a diet with a low ω -6/ ω -3 ratio from P2 to P42 has lasting benefits for cognitive function. This relatively short exposure to a diet with low ω -6/ ω -3 counteracted the negative effects of ES in OR, OL, and MWM performance but did not rescue the ES-induced metabolic alterations. This indicates that the ES-induced alterations in FA during this critical developmental period might be a determinant factor in the cognitive impairments observed in adulthood. Furthermore, the diet did not alter maternal care and markers of synaptic plasticity or apoptosis but prevented the ES-induced alterations in hippocampal cell survival and microglial CD68 expression in adulthood, indicating that these processes might underlie the beneficial effects of early diet on cognition.

Diet with low ω -6/ ω -3 ratio protects against ES-induced cognitive impairments

In our study, ES did not alter the hepatic TG content or lipid metabolism-related gene expression. However, ES affected the FA profile in the offspring liver and hippocampus at P9, suggesting that ES might have induced changes in offspring FA uptake, synthesis, and metabolism (64–67).

Interestingly, although ES reduced the ω -6/ ω -3 ratio in the liver at P9, suggestive of a higher ω -3 bioavailability, the hippocampus showed opposite effects (*e.g.*, reductions in total PUFA content, mostly because of a reduction in the 2 most abundant LCPUFA in the brain: DHA and AA).

Considering that DHA is highly accreted in the rodent brain during the first weeks of postnatal life (14, 68) and ω -3 PUFA deficiencies during this early period are associated with cognitive and emotional disorders in adulthood (14, 15, 69–72), our data suggest that early reduction in central PUFA after ES is involved in programming adult cognitive functions.

In adulthood, ES reduced ω -6/ ω -3 ratio in the liver and increased hippocampal LCPUFA (AA and DHA). Despite this apparent “beneficial” LCPUFA profile, adult ES mice exhibit cognitive impairments, suggesting that mechanisms other than adult brain FA status mediate cognitive functions after ES. Possibly, the adult PUFA levels could be a compensatory mechanism, though insufficient to prevent the cognitive impairments. Alternatively, there is evidence that inflammation (*e.g.*, induced by LPS) can increase level of PUFA in the brain, particularly released by astrocytes (73). Considering that ES leads to a more proinflammatory profile in the adult brain (54), this might possibly stimulate increased release of PUFA by the astrocytes and lead to the observed increase in ES-induced PUFA in the brain.

We have further demonstrated that low dietary ω -6/ ω -3 early in life prevented ES-induced impairments. ES effects on hippocampal FA profiles were similar between mice under standard grain-based chow conditions (study 1) and those on high ω -6/ ω -3 diet (study 2) at P9. Later in life, there were no effects of ES at P180 in mice previously fed high or low ω -6/ ω -3 diets in contrast with the standard grain-based diet, which might be related to subtle differences in nutritional composition between diets. However, under both diet conditions, the ES paradigm led to similar cognitive impairments. Importantly, the low ω -6/ ω -3 diet prevented these ES-induced reductions in DHA at P9, suggesting that the restored early-life DHA status might

(at least partly) mediate the ES-induced cognitive impairments in adulthood.

Although the low ω -6/ ω -3 diet prevented the ES-induced cognitive impairments, it did not affect adiposity and leptin levels in CTL and ES mice in the short and long term. Possible protective effects of the diet on the ES-induced metabolic phenotype might be more apparent under metabolic challenges (e.g., Western-style diet exposure). Indeed, it has been shown that early-life exposure to a reduced ω -6/ ω -3 diet protects against excessive body fat accumulation caused by adult Western-style diet exposure (20, 43). Also, we previously showed that ES reduced adiposity in mice on standard diet, whereas it increased body fat accumulation when mice were challenged with a moderate Western-style diet (20% fat) in adulthood (2).

Taken together, the low ω -6/ ω -3 diet-induced improvements in hippocampal FA profile during early development might be instrumental for hippocampal function later in life and contribute to the prevention of the ES-induced cognitive deficits in adulthood. This supports the concept that the developmental central FA status, determined by diet, is key for later brain function.

Potential mechanisms underlying the beneficial effects of the low ω -6/ ω -3 diet on ES-induced cognitive impairments

We assessed several possible processes potentially contributing to the beneficial effects of low ω -6/ ω -3 diet on cognition after ES. We replicated the finding that similar to grain-based diet, ES reduced survival of newborn cells under the high ω -6/ ω -3 diet and demonstrated that the low ω -6/ ω -3 diet prevented the ES-induced reductions in hippocampal cell survival. Our findings are in line with existing reports showing stimulating effects of increased ω -3 PUFA on adult hippocampal neurogenesis (31, 74–76) and support the notion that neurogenesis contributes to the beneficial effect of the low ω -6/ ω -3 diet in preventing the ES-induced cognitive impairments.

As an additional possible mechanism, we studied microglia and reproduced our recent findings of ES, increasing the number of CD68⁺ cells, a marker for immunoreactive phagocytic microglia (77), in the hippocampus of adult mice exposed to the high ω -6/ ω -3 diet (54). Importantly, this effect was prevented by the low ω -6/ ω -3 diet, suggesting that, in line with the anti-inflammatory properties of ω -3 FA (19, 78), increased ω -3 status early in life suppresses the ES-induced increase in the microglial phagocytic marker CD68, possibly mediating the improvement in cognition. This is further supported by the negative correlation between hippocampal learning and CD68 expression. Additionally, microglia might also contribute to the changes in neurogenesis because, among their many functions, microglia play critical roles in synaptic tuning and pruning of newborn neurons (79–81), thereby contributing to the beneficial effects on cognition after ES. Contributing to the cognitive impairments as well as the alterations in the neuroinflammation that we observe after ES and diet, inflammatory changes and oxidative mechanisms in the circulatory and gastrointestinal systems may be involved as well (82).

In the current study, we further demonstrated that hippocampal *Bdnf* gene expression was reduced by the low ω -6/ ω -3 diet at P9 and transiently increased by ES at P42. This is in contrast with other studies showing decreased *Bdnf* protein levels in the hippocampus and prefrontal cortex after ES in later life (57, 83). Moreover, we only found ES-induced alterations on maternal care, body weight, and food intake as well as in the adult hippocampal Bax/Bcl2 ratio in offspring without further diet effects.

Taken together, we show that ES affects central and peripheral FA composition, and a relatively subtle modulation of PUFA composition (e.g., a low ω -6/ ω -3 ratio in the diet) during the early sensitive period provides protection against the adverse lasting effects of ES on later cognition that involves modulations of hippocampal cell survival and microglia. Our study highlights the relevance of early nutrition for later brain functions and hence as a powerful and promising tool for possible future interventions in vulnerable populations exposed to stress during early life. FJ

ACKNOWLEDGMENTS

A.K. is supported by JPI CogniPlast, Nederlands Organisatie voor Wetenschappelijk Onderzoek (NWO)-Meervoud, and the NWO Food, Cognition, and Brain Grant. P.J.L. is supported by Alzheimer Nederland. This work received cofunding from Danone Nutricia Research, employer of L.S., A.O., and E.M.V.D.B. The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

K.-Y. Yam, L. Schipper, and A. Korosi conceived and designed the study; L. Schipper, A. Oosting, and E. M. Van der Beek designed and developed the specific diets and guided the metabolic aspects of the study; K.-Y. Yam performed the experiments and analysis; K. Reemst, S. R. Ruigrok, M. R. Abbink, L. Hoeijmakers, E. F. G. Naninck, and P. Zarekiani contributed to the acquisition of the data; K.-Y. Yam, L. Schipper, and A. Korosi made substantial contributions to the interpretation of the data; P.J. Lucassen critically read the manuscript; and K.-Y. Yam and A. Korosi wrote the manuscript with substantial contribution by all other authors.

REFERENCES

1. Naninck, E. F., Hoeijmakers, L., Kakava-Georgiadou, N., Meesters, A., Lazic, S. E., Lucassen, P. J., and Korosi, A. (2015) Chronic early life stress alters developmental and adult neurogenesis and impairs cognitive function in mice. *Hippocampus* **25**, 309–328.
2. Yam, K. Y., Naninck, E. F. G., Abbink, M. R., la Fleur, S. E., Schipper, L., van den Beukel, J. C., Grefhorst, A., Oosting, A., van der Beek, E. M., Lucassen, P. J., and Korosi, A. (2017) Exposure to chronic early-life stress lastingly alters the adipose tissue, the leptin system and changes the vulnerability to Western-style diet later in life in mice. *Psychoneuroendocrinology* **77**, 186–195.
3. Chugani, H. T., Behen, M. E., Muzik, O., Juhász, C., Nagy, F., and Chugani, D. C. (2001) Local brain functional activity following early deprivation: a study of postinstitutionalized Romanian orphans. *Neuroimage* **14**, 1290–1301.
4. Murphy, M. O., and Loria, A. S. (2017) Sex-specific effects of stress on metabolic and cardiovascular disease: are women at higher risk? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **313**, R1–R9.

5. Korosi, A., and Baram, T. Z. (2009) The pathways from mother's love to baby's future. *Front. Behav. Neurosci.* **3**, 27
6. Walker, C. D. (2010) Maternal touch and feed as critical regulators of behavioral and stress responses in the offspring. *Dev. Psychobiol.* **52**, 638–650
7. Walker, C. D. (2005) Nutritional aspects modulating brain development and the responses to stress in early neonatal life. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **29**, 1249–1263
8. Lucassen, P. J., Naninck, E. F., van Goudoever, J. B., Fitzsimons, C., Joels, M., and Korosi, A. (2013) Perinatal programming of adult hippocampal structure and function; emerging roles of stress, nutrition and epigenetics. *Trends Neurosci.* **36**, 621–631
9. Naninck, E. F., Oosterink, J. E., Yam, K. Y., de Vries, L. P., Schierbeek, H., van Goudoever, J. B., Verkaik-Schakel, R. N., Plantinga, J. A., Plosch, T., Lucassen, P. J., and Korosi, A. (2017) Early micronutrient supplementation protects against early stress-induced cognitive impairments. *FASEB J.* **31**, 505–518
10. Maniam, J., Antoniadis, C., and Morris, M. J. (2014) Early-life stress, HPA axis adaptation, and mechanisms contributing to later health outcomes. *Front. Endocrinol. (Lausanne)* **5**, 73
11. Spencer, S. J. (2013) Perinatal nutrition programs neuroimmune function long-term: mechanisms and implications. *Front. Neurosci.* **7**, 144
12. Innis, S. M. (2008) Dietary omega 3 fatty acids and the developing brain. *Brain Res.* **1237**, 35–43
13. Pusceddu, M. M., Kelly, P., Stanton, C., Cryan, J. F., and Dinan, T. G. (2016) N-3 polyunsaturated fatty acids through the lifespan: implication for psychopathology. *Int. J. Neuropsychopharmacol.* **19**
14. McNamara, R. K., and Carlson, S. E. (2006) Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins Leukot. Essent. Fatty Acids* **75**, 329–349
15. Joffe, C., Nadjar, A., Lebbadi, M., Calon, F., and Laye, S. (2014) n-3 LCPUFA improves cognition: the young, the old and the sick. *Prostaglandins Leukot. Essent. Fatty Acids* **91**, 1–20
16. Brenna, J. T. (2011) Animal studies of the functional consequences of suboptimal polyunsaturated fatty acid status during pregnancy, lactation and early post-natal life. *Matern. Child Nutr.* **7** (Suppl 2), 59–79
17. Janssen, C. I., Zerbi, V., Mutsaers, M. P., de Jong, B. S., Wiesmann, M., Arnoldussen, I. A., Geenen, B., Heerschap, A., Muskiet, F. A., Jouni, Z. E., van Tol, E. A., Gross, G., Homberg, J. R., Berg, B. M., and Kiliaan, A. J. (2015) Impact of dietary n-3 polyunsaturated fatty acids on cognition, motor skills and hippocampal neurogenesis in developing C57BL/6J mice. *J. Nutr. Biochem.* **26**, 24–35
18. Robertson, R. C., Seira Oriach, C., Murphy, K., Moloney, G. M., Cryan, J. F., Dinan, T. G., Paul Ross, R., and Stanton, C. (2017) Omega-3 polyunsaturated fatty acids critically regulate behaviour and gut microbiota development in adolescence and adulthood. *Brain Behav. Immun.* **59**, 21–37
19. Dalli, J., and Serhan, C. N. (2018) Immunoresolvents signaling molecules at intersection between the brain and immune system. *Curr. Opin. Immunol.* **50**, 48–54
20. Oosting, A., Kegler, D., Boehm, G., Jansen, H. T., van de Heijning, B. J., and van der Beek, E. M. (2010) N-3 long-chain polyunsaturated fatty acids prevent excessive fat deposition in adulthood in a mouse model of postnatal nutritional programming. *Pediatr. Res.* **68**, 494–499
21. Muhlhauser, B. S., Miljkovic, D., Fong, L., Xian, C. J., Duthoit, E., and Gibson, R. A. (2011) Maternal omega-3 supplementation increases fat mass in male and female rat offspring. *Front. Genet.* **2**, 48
22. Mennitti, L. V., Oliveira, J. L., Morais, C. A., Estadella, D., Oyama, L. M., Oller do Nascimento, C. M., and Pisani, L. P. (2015) Type of fatty acids in maternal diets during pregnancy and/or lactation and metabolic consequences of the offspring. *J. Nutr. Biochem.* **26**, 99–111
23. Korotkova, M., Gabrielson, B., Hanson, L. A., and Strandvik, B. (2001) Maternal essential fatty acid deficiency depresses serum leptin levels in suckling rat pups. *J. Lipid Res.* **42**, 359–365
24. Jen, K. L., Church, M. W., Wang, C., Moghaddam, M., Dowhan, L., Laja, F., and Sherman, J. (2009) Perinatal n-3 fatty acid imbalance affects fatty acid composition in rat offspring. *Physiol. Behav.* **98**, 17–24
25. Sardinha, F. L., Fernandes, F. S., Tavares do Carmo, M. G., and Herrera, E. (2013) Sex-dependent nutritional programming: fish oil intake during early pregnancy in rats reduces age-dependent insulin resistance in male, but not female, offspring. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **304**, R313–R320
26. Lombardo, Y. B., and Chicco, A. G. (2006) Effects of dietary polyunsaturated n-3 fatty acids on dyslipidemia and insulin resistance in rodents and humans. A review. *J. Nutr. Biochem.* **17**, 1–13
27. Delarue, J., LeFoll, C., Corporeau, C., and Lucas, D. (2004) N-3 long chain polyunsaturated fatty acids: a nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity? *Reprod. Nutr. Dev.* **44**, 289–299
28. Gibson, R. A., Muhlhauser, B., and Makrides, M. (2011) Conversion of linoleic acid and alpha-linolenic acid to long-chain polyunsaturated fatty acids (LCPUFAs), with a focus on pregnancy, lactation and the first 2 years of life. *Matern. Child Nutr.* **7** (Suppl 2), 17–26
29. Delpech, J. C., Thomazeau, A., Madore, C., Bosch-Bouju, C., Larrieu, T., Lacabanne, C., Remus-Borel, J., Aubert, A., Joffe, C., Nadjar, A., and Layé, S. (2015) Dietary n-3 PUFAs deficiency increases vulnerability to inflammation-induced spatial memory impairment. *Neuropsychopharmacology* **40**, 2774–2787
30. Harauma, A., and Moriguchi, T. (2011) Dietary n-3 fatty acid deficiency in mice enhances anxiety induced by chronic mild stress. *Lipids* **46**, 409–416
31. Fan, C., Fu, H., Dong, H., Lu, Y., Lu, Y., and Qi, K. (2016) Maternal n-3 polyunsaturated fatty acid deprivation during pregnancy and lactation affects neurogenesis and apoptosis in adult offspring: associated with DNA methylation of brain-derived neurotrophic factor transcripts. *Nutr. Res.* **36**, 1013–1021
32. Larrieu, T., Hilal, M. L., Fourier, C., De Smedt-Peyrusse, V., Sans, N., Capuron, L., and Layé, S. (2014) Nutritional omega-3 modulates neuronal morphology in the prefrontal cortex along with depression-related behaviour through corticosterone secretion. *Transl. Psychiatry* **4**, e437; erratum: e468
33. Venna, V. R., Deplanque, D., Allet, C., Belarbi, K., Hamdane, M., and Bordet, R. (2009) PUFA induce antidepressant-like effects in parallel to structural and molecular changes in the hippocampus. *Psychoneuroendocrinology* **34**, 199–211
34. Schipper, L., Oosting, A., Scheurink, A. J. W., van Dijk, G., and van der Beek, E. M. (2016) Reducing dietary intake of linoleic acid of mouse dams during lactation increases offspring brain n-3 LCPUFA content. *Prostaglandins Leukot. Essent. Fatty Acids* **110**, 8–15
35. Simopoulos, A. P. (2009) Evolutionary aspects of the dietary omega-6: omega-3 fatty acid ratio: medical implications. *World Rev. Nutr. Diet.* **100**, 1–21
36. Yehuda, S. (2003) Omega-6/omega-3 ratio and brain-related functions. *World Rev. Nutr. Diet.* **92**, 37–56
37. Simopoulos, A. P., Leaf, A., and Salem, N., Jr. (1999) Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Ann. Nutr. Metab.* **43**, 127–130
38. Simopoulos, A. P. (2002) The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.* **56**, 365–379
39. Jumpsen, J., Lien, E. L., Goh, Y. K., and Clandinin, M. T. (1997) Small changes of dietary (n-6) and (n-3)/fatty acid content ration alter phosphatidylethanolamine and phosphatidylcholine fatty acid composition during development of neuronal and glial cells in rats. *J. Nutr.* **127**, 724–731
40. Sakayori, N., Tokuda, H., Yoshizaki, K., Kawashima, H., Innis, S. M., Shibata, H., and Osumi, N. (2016) Maternal nutritional imbalance between linoleic acid and alpha-linolenic acid increases offspring's anxious behavior with a sex-dependent manner in mice. *Tohoku J. Exp. Med.* **240**, 31–37
41. Tian, C., Fan, C., Liu, X., Xu, F., and Qi, K. (2011) Brain histological changes in young mice submitted to diets with different ratios of n-6/n-3 polyunsaturated fatty acids during maternal pregnancy and lactation. *Clin. Nutr.* **30**, 659–667
42. Ailhaud, G., Massiera, F., Weill, P., Legrand, P., Alessandri, J. M., and Guesnet, P. (2006) Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. *Prog. Lipid Res.* **45**, 203–236
43. Oosting, A., Kegler, D., van de Heijning, B. J., Verkade, H. J., and van der Beek, E. M. (2015) Reduced linoleic acid intake in early postnatal life improves metabolic outcomes in adult rodents following a Western-style diet challenge. *Nutr. Res.* **35**, 800–811
44. Clarke, G., O'Mahony, S. M., Hennessy, A. A., Ross, P., Stanton, C., Cryan, J. F., and Dinan, T. G. (2009) Chain reactions: early-life stress alters the metabolic profile of plasma polyunsaturated fatty acids in adulthood. *Behav. Brain Res.* **205**, 319–321
45. Bernardi, J. R., Ferreira, C. F., Senter, G., Krolow, R., de Aguiar, B. W., Portella, A. K., Kauer-Sant'anna, M., Kapczinski, F., Dalmaz, C., Goldani, M. Z., and Silveira, P. P. (2013) Early life stress interacts with the diet deficiency of omega-3 fatty acids during the life course increasing the metabolic vulnerability in adult rats. *PLoS One* **8**, e62031
46. Mathieu, G., Denis, S., Lavialle, M., and Vancassel, S. (2008) Synergistic effects of stress and omega-3 fatty acid deprivation on

- emotional response and brain lipid composition in adult rats. *Prostaglandins Leukot. Essent. Fatty Acids* **78**, 391–401
47. Ferreira, C. F., Bernardi, J. R., Krolow, R., Arcego, D. M., Fries, G. R., de Aguiar, B. W., Senter, G., Kapczinski, F. P., Silveira, P. P., and Dalmaz, C. (2013) Vulnerability to dietary n-3 polyunsaturated fatty acid deficiency after exposure to early stress in rats. *Pharmacol. Biochem. Behav.* **107**, 11–19
 48. Mathieu, G., Oualian, C., Denis, I., Lavialle, M., Gisquet-Verrier, P., and Vancassel, S. (2011) Dietary n-3 polyunsaturated fatty acid deprivation together with early maternal separation increases anxiety and vulnerability to stress in adult rats. *Prostaglandins Leukot. Essent. Fatty Acids* **85**, 129–136
 49. Dutta, S., and Sengupta, P. (2016) Men and mice: relating their ages. *Life Sci.* **152**, 244–248
 50. Diniz, B. S., Teixeira, A. L., Campos, A. C., Miranda, A. S., Rocha, N. P., Talib, L. L., Gattaz, W. F., and Forlenza, O. V. (2012) Reduced serum levels of adiponectin in elderly patients with major depression. *J. Psychiatr. Res.* **46**, 1081–1085
 51. Farr, O. M., Tsoukas, M. A., and Mantzoros, C. S. (2015) Leptin and the brain: influences on brain development, cognitive functioning and psychiatric disorders. *Metabolism* **64**, 114–130
 52. Guo, M., Huang, T. Y., Garza, J. C., Chua, S. C., and Lu, X. Y. (2013) Selective deletion of leptin receptors in adult hippocampus induces depression-related behaviours. *Int. J. Neuropsychopharmacol.* **16**, 857–867
 53. Milaneschi, Y., Lamers, F., Bot, M., Drent, M. L., and Penninx, B. W. (2017) Leptin dysregulation is specifically associated with major depression with atypical features: evidence for a mechanism connecting obesity and depression. *Biol. Psychiatry* **81**, 807–814
 54. Hoeijmakers, L., Ruijgrok, S. R., Amelanchik, A., Ivan, D., van Dam, A. M., Lucassen, P. J., and Korosi, A. (2017) Early-life stress lastingly alters the neuroinflammatory response to amyloid pathology in an Alzheimer's disease mouse model. *Brain Behav. Immun.* **63**, 160–175
 55. Abbink, M. R., Naninck, E. F. G., Lucassen, P. J., and Korosi, A. (2017) Early-life stress diminishes the increase in neurogenesis after exercise in adult female mice. *Hippocampus* **27**, 839–844
 56. Lajud, N., and Torner, L. (2015) Early life stress and hippocampal neurogenesis in the neonate: sexual dimorphism, long term consequences and possible mediators. *Front. Mol. Neurosci.* **8**, 3
 57. Rincel, M., Lépinay, A. L., Delage, P., Fioramonti, J., Théodorou, V. S., Layé, S., and Darnaudéry, M. (2016) Maternal high-fat diet prevents developmental programming by early-life stress. *Transl. Psychiatry* **6**, e966
 58. Loi, M., Koricka, S., Lucassen, P. J., and Joëls, M. (2014) Age- and sex-dependent effects of early life stress on hippocampal neurogenesis. *Front. Endocrinol. (Lausanne)* **5**, 13
 59. Rice, C. J., Sandman, C. A., Lenjavi, M. R., and Baram, T. Z. (2008) A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology* **149**, 4892–4900
 60. Reeves, P. G., Nielsen, F. H., and Fahey, G. C., Jr. (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **123**, 1939–1951
 61. Deacon, R. M., and Rawlins, J. N. (2006) T-maze alternation in the rodent. *Nat. Protoc.* **1**, 7–12
 62. Bligh, E. G., and Dyer, W. J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911–917
 63. Guilloux, J. P., Seney, M., Edgar, N., and Sibille, E. (2011) Integrated behavioral z-scoring increases the sensitivity and reliability of behavioral phenotyping in mice: relevance to emotionality and sex. *J. Neurosci. Methods* **197**, 21–31
 64. Choi, S. S., and Diehl, A. M. (2008) Hepatic triglyceride synthesis and nonalcoholic fatty liver disease. *Curr. Opin. Lipidol.* **19**, 295–300
 65. El-Badry, A. M., Graf, R., and Clavien, P. A. (2007) Omega 3-omega 6: what is right for the liver? *J. Hepatol.* **47**, 718–725
 66. Scorletti, E., and Byrne, C. D. (2013) Omega-3 fatty acids, hepatic lipid metabolism, and nonalcoholic fatty liver disease. *Annu. Rev. Nutr.* **33**, 231–248
 67. Rapoport, S. I., Rao, J. S., and Igarashi, M. (2007) Brain metabolism of nutritionally essential polyunsaturated fatty acids depends on both the diet and the liver. *Prostaglandins Leukot. Essent. Fatty Acids* **77**, 251–261
 68. Green, P., and Yavin, E. (1998) Mechanisms of docosahexaenoic acid accretion in the fetal brain. *J. Neurosci. Res.* **52**, 129–136
 69. Carlson, S. E., and Neuringer, M. (1999) Polyunsaturated fatty acid status and neurodevelopment: a summary and critical analysis of the literature. *Lipids* **34**, 171–178
 70. Lauritzen, L., Brambilla, P., Mazzocchi, A., Harsløf, L. B., Ciappolino, V., and Agostoni, C. (2016) DHA effects in brain development and function. *Nutrients* **8**
 71. Madore, C., Nadjar, A., Delpech, J. C., Sere, A., Aubert, A., Portal, C., Joffre, C., and Layé, S. (2014) Nutritional n-3 PUFAs deficiency during perinatal periods alters brain innate immune system and neuronal plasticity-associated genes. *Brain Behav. Immun.* **41**, 22–31
 72. Salem, N., Jr., Moriguchi, T., Greiner, R. S., McBride, K., Ahmad, A., Catalan, J. N., and Slotnick, B. (2001) Alterations in brain function after loss of docosahexaenoate due to dietary restriction of n-3 fatty acids. *J. Mol. Neurosci.* **16**, 299–307, discussion 317–321
 73. Aizawa, F., Nishinaka, T., Yamashita, T., Nakamoto, K., Koyama, Y., Kasuya, F., and Tokuyama, S. (2016) Astrocytes release polyunsaturated fatty acids by lipopolysaccharide stimuli. *Biol. Pharm. Bull.* **39**, 1100–1106
 74. Coti Bertrand, P., O'Kusky, J. R., and Innis, S. M. (2006) Maternal dietary (n-3) fatty acid deficiency alters neurogenesis in the embryonic rat brain. *J. Nutr.* **136**, 1570–1575
 75. Niculescu, M. D., Lupu, D. S., and Craciunescu, C. N. (2011) Maternal α -linolenic acid availability during gestation and lactation alters the postnatal hippocampal development in the mouse offspring. *Int. J. Dev. Neurosci.* **29**, 795–802
 76. He, C., Qu, X., Cui, L., Wang, J., and Kang, J. X. (2009) Improved spatial learning performance of fat-1 mice is associated with enhanced neurogenesis and neuritogenesis by docosahexaenoic acid. *Proc. Natl. Acad. Sci. USA* **106**, 11370–11375
 77. Chistiakov, D. A., Killingsworth, M. C., Myasoedova, V. A., Orekhov, A. N., and Bobryshev, Y. V. (2017) CD68/macrosialin: not just a histochemical marker. *Lab. Invest.* **97**, 4–13
 78. Orr, S. K., Trépanier, M. O., and Bazinet, R. P. (2013) n-3 polyunsaturated fatty acids in animal models with neuroinflammation. *Prostaglandins Leukot. Essent. Fatty Acids* **88**, 97–103
 79. Ekdahl, C. T. (2012) Microglial activation - tuning and pruning adult neurogenesis. *Front. Pharmacol.* **3**, 41
 80. Paolicelli, R. C., Bolasco, G., Pagani, F., Maggi, L., Scianni, M., Panzanelli, P., Giustetto, M., Ferreira, T. A., Guiducci, E., Dumas, L., Ragozzino, D., and Gross, C. T. (2011) Synaptic pruning by microglia is necessary for normal brain development. *Science* **333**, 1456–1458
 81. Rey, C., Nadjar, A., Joffre, F., Amadiou, C., Aubert, A., Vaysse, C., Pallet, V., Layé, S., and Joffre, C. (2018) Maternal n-3 polyunsaturated fatty acid dietary supply modulates microglia lipid content in the offspring. *Prostaglandins Leukot. Essent. Fatty Acids* **133**, 1–7
 82. Wohleb, E. S., and Delpech, J. C. (2017) Dynamic cross-talk between microglia and peripheral monocytes underlies stress-induced neuroinflammation and behavioral consequences. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **79** (Pt A), 40–48
 83. Roceri, M., Hendriks, W., Racagni, G., Ellenbroek, B. A., and Riva, M. A. (2002) Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. *Mol. Psychiatry* **7**, 609–616

Received for publication November 5, 2018.

Accepted for publication January 2, 2019.